D5

(19) Europäisches Patentamt
European Patent Office
Office europ´en des brevets

(11) Publication number: **0 426 857 A1**

(12) **EUROPEAN PATENT APPLICATION published in accordance with Art. 158(3) EPC**

(21) Application number: **90902687.4**

(22) Date of filing: **06.02.90**

(86) International application number: **PCT/JP90/00142**

(87) International publication number: **WO 90/09396 (23.08.90 90/20)**

(51) Int. Cl.5: **C07K 7/08**, C07K 7/10, C07K 17/08, C07K 17/10, C07K 17/14, A61K 37/02, B01J 20/22, G01N 33/564, G01N 33/566

(30) Priority: **08.02.89 JP 130738/89**
18.10.89 JP 272254/89

(43) Date of publication of application: **15.05.91 Bulletin 91/20**

(84) Designated Contracting States: **DE FR GB IT**

(71) Applicant: **KURARAY Co. LTD.**
1621 Sakazu Kurashiki-shi
Okayama 710(JP)

(72) Inventor: **TANIHARA, Masao**
1625, Sakazu Kurashiki-shi
Okayama 710(JP)
Inventor: **OKA, Kiichiro**
2047-1, Sakazu Kurashiki-shi
Okayama 710(JP)

(74) Representative: **Vossius & Partner**
Siebertstrasse 4 P.O. Box 86 07 67
W-8000 München 86(DE)

(54) **PEPTIDE AND ADSORBENT COMPRISING SAME IMMOBILIZED ON CARRIER.**

(57) This invention relates to a peptide capable of combining with interleukin 6 and an adsorbent for interleukin 6 comprising said peptide immobilized on a carrier.

EP 0 426 857 A1

PEPTIDE AND ADSORBENT THEREOF IMMOBILIZED ON CARRIER

FIELD OF THE INVENTION

The present invention relates to a peptide being capable of binding to interleukin 6, and an adsorbent for interleukin 6 comprising the peptide immobilized on a carrier.

It is known that interleukin 6 (hereinafter abbreviated as IL-6) acts on lymphocytes which are capable of producing an antibody to remarkably enhance productivity of the antibody, and IL-6 is considered to be one of causative agents of autoimmune diseases such as rheumatism and the like. Accordingly, the peptide and the adsorbent of the present invention are useful for treatment of autoimmune diseases such as rheumatism and the like.

PRIOR ART

Science, Vol. 241, pages 825 to 828 (1988) reports that a precursor of human interleukin 6 receptor (hereinafter abbreviated as IL-6 receptor) is composed of 468 amino acids and its primary structure has been elucidated. According to this report, the primary structure of a mature type IL-6 receptor is represented by the formula:

Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg
Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu
Thr Cys Pro Gly Val Glu Pro Glu Asp Asn Ala Thr Val
His Trp Val Leu Arg Lys Pro Ala Ala Gly Ser His Pro
Ser Arg Trp Ala Gly Met Gly Arg Arg Leu Leu Leu Arg
Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys Tyr
Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val
Asp Val Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg
Lys Ser Pro Leu Ser Asn Val Val Cys Glu Trp Gly Pro
Arg Ser Thr Pro Ser Leu Thr Thr Lys Ala Val Leu Leu
Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp Phe Gln
Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser
Cys Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr
Ile Val Ser Met Cys Val Ala Ser Ser Val Gly Ser Lys
Phe Ser Lys Thr Gln Thr Phe Gln Gly Cys Gly Ile Leu
Gln Pro Asp Pro Pro Ala Asn Ile Thr Val Thr Ala Val
Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp
Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe
Glu Leu Arg Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr
Thr Trp Met Val Lys Asp Leu Gln His His Cys Val Ile
His Asp Ala Trp Ser Gly Leu Arg His Val Val Gln Leu
Arg Ala Gln Glu Glu Phe Gly Gln Gly Glu Trp Ser Glu
Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser

Arg Ser Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met
Gln Ala Leu Thr Thr Asn Lys Asp Asp Asp Asn Ile Leu
Phe Arg Asp Ser Ala Asn Ala Thr Ser Leu Pro Val Gln
Asp Ser Ser Ser Val Pro Leu Pro Thr Phe Leu Val Ala
Gly Gly Ser Leu Ala Phe Gly Thr Leu Leu Cys Ile Ala
Ile Val Leu Arg Phe Lys Lys Thr Trp Lys Leu Arg Ala
Leu Lys Glu Gly Lys Thr Ser Met His Pro Pro Tyr Ser
Leu Gly Gln Leu Val Pro Glu Arg Pro Arg Pro Thr Pro
Val Leu Val Pro Leu Ile Ser Pro Pro Val Ser Pro Ser
Ser Leu Gly Ser Asp Asn Thr Ser Ser His Asn Arg Pro
Asp Ala Arg Asp Pro Arg Ser Pro Tyr Asp Ile Ser Asn
Thr Asp Tyr Phe Phe Pro Arg

Further, Medical Immunology, Vol. 15, pages 195 to 201 (1988) discloses a report of a relation between IL-6 and autoimmune diseases.

In treatment of autoimmune diseases such as rheumatism and the like, it has been requested to establish means for removing IL-6 which is considered to be a main causative agent of such diseases. However, any practical method thereof has not yet been established.

One object of the present invention is to provide a novel peptide being capable of binding to IL-6. Another object of the present invention is to provide an adsorbent for IL-6 comprising the novel peptide immobilized on a carrier.

DISCLOSURE OF THE INVENTION

According to the present invention, there is provided (1) a peptide being capable of binding to IL-6 represented by the general formula:

$$H-X-A-Y-Z \qquad [I]$$

[wherein A is a peptide segment formed by binding 6 to 50 amino acids; each of X and Y is a single bond or an amino acid residue selected from the group consisting of Asp, Glu, Lys, Ala and a divalent group of the formula: $-NH(CH_2)_n-CO-$(wherein n is an integer of 1 to 17), or a peptide segment composed of 2 to 10 amino acid residues selected from the above group which are bound to each other through a peptide bond; Z is hydroxyl group or amino group]. Further, according to the present invention, there is provided (2) an adsorbent comprising the peptide immobilized on a carrier.

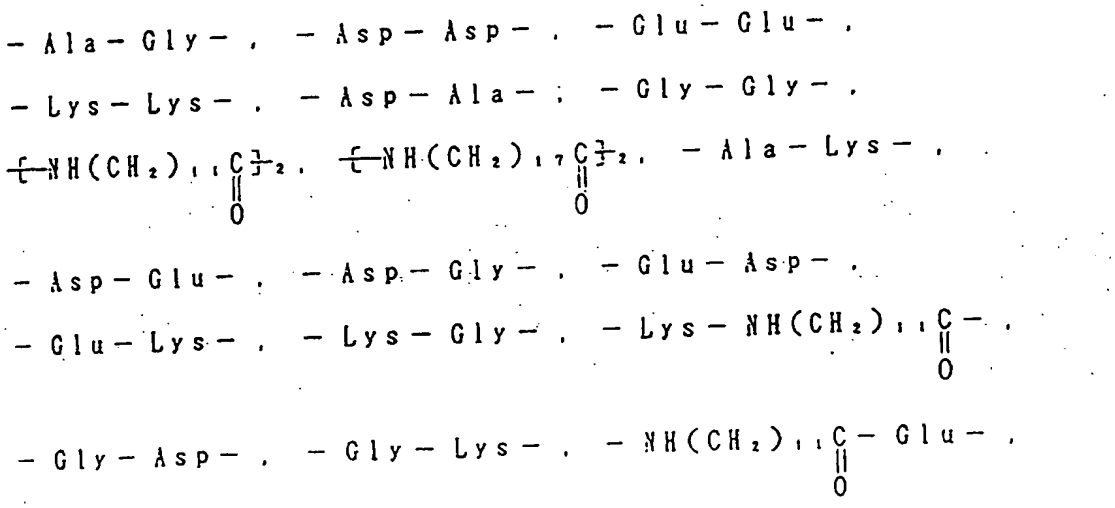
In the present specification, various amino acid residues are abbreviated as follows:

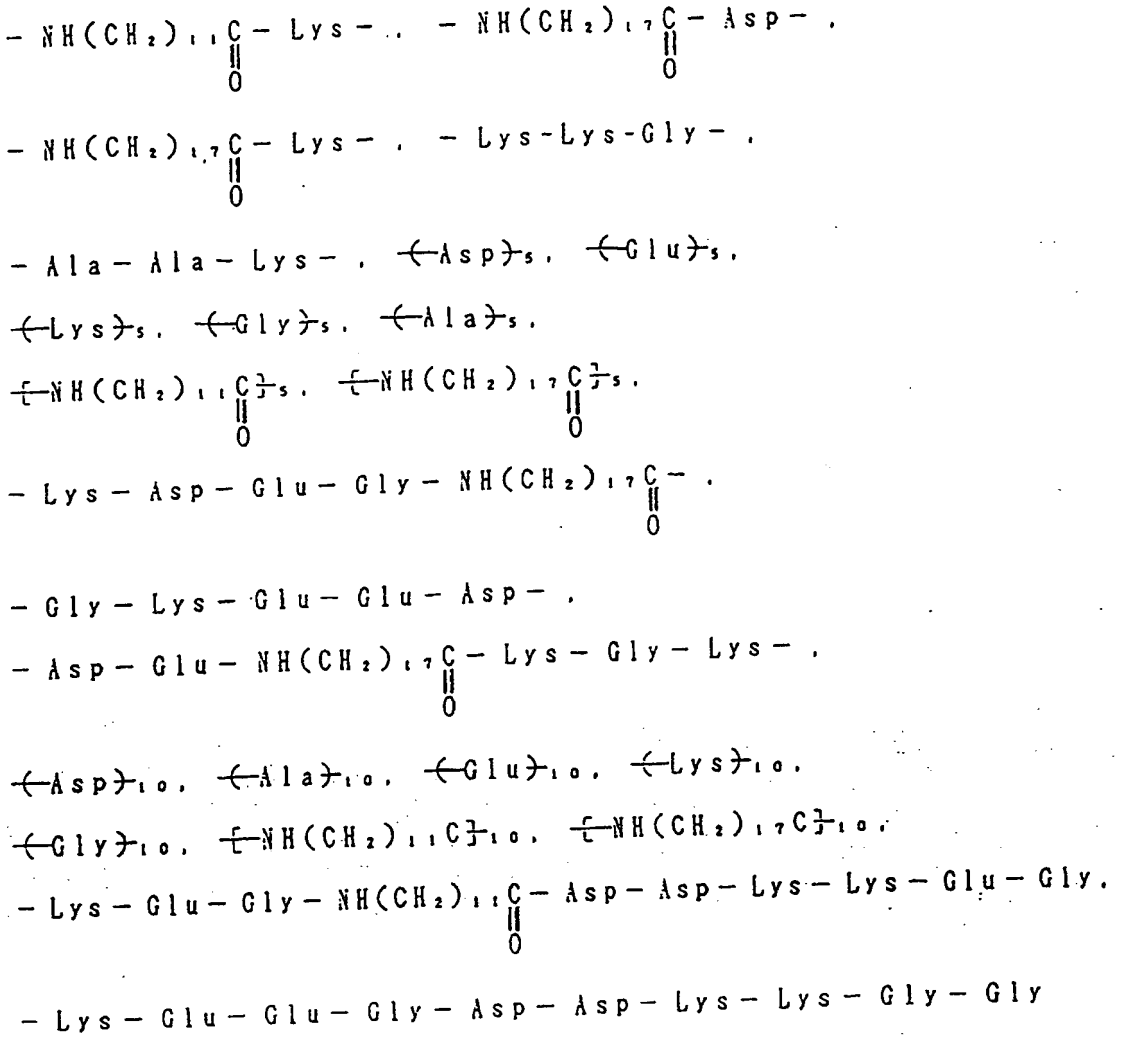
Ala: L-alanine residue,
Arg: L-arginine residue,
Asn: L-asparagine residue,
Asp: L-aspartic acid residue,
Cys: L-cysteine residue,
Gln: L-glutamine residue,

Glu: L-glutamic acid residue,
Gly: glycine residue,
His: L-histidine residue,
Ile: L-isoleucine residue,
Leu: L-leucine residue,
Lys: L-lysine residue,
Phe: L-phenylalanine residue,
Pro: L-proline residue,
Ser: L-serine residue,
Thr: L-threonine residue,
Trp: L-tryptophan residue,
Tyr: L-tyrosine residue,
Val: L-valine residue.

Further, in the present specification, the amino acid sequence is described in such a manner that the amino acid residue at the N-terminal is located on the left hand and the amino acid residue at the C-terminal is located on the right hand according to the conventional method.

As the peptide segment represented by X and Y in the general formula (I), for Example, there are the following peptide segments:





When the peptide represented by the general formula (I) wherein X and/or Y are peptide segments composed of 11 or more amino acid residues selected from the above group which are bound to each other through a peptide bond, such a peptide may not have ability to binding to the desired IL-6.

Suitable examples of the peptide segment represented by A in the general formula (I) are as follows. The amino acid residues of each peptide segment may be those subjected to homologous substitution.

(a) – Thr – Ser – Leu – Pro – Gly – Asp – Ser – Val – Thr – Leu – Thr – Cys – Pro – Gly – Val – Glu – Pro – Glu – Asp –

(b) – Gly – Thr – Val – His – Leu – Leu – Val – Asp – Val – Pro – Pro – Glu – Glu – Pro – Gln – Leu – Ser – Cys – Phe – Arg – Lys –

(c) – Ser – Thr – Pro – Ser – Leu – Thr – Thr – Lys – Ala – Val – Leu – Leu – Val – Arg – Lys – Phe – Gln – Asn – Ser – Pro – Ala – Glu – Asp –

(d) – Arg – Lys – Phe – Gln – Asn – Ser – Pro – Ala – Glu – Asp – Phe – Gln – Glu – Pro – Cys – Gln – Tyr – Ser – Gln – Glu – Ser –

(e) – Asn – Pro – Arg – Trp – Leu – Ser – Val – Thr – Trp – Gln – Asp – Pro – His – Ser –

(f) – Trp – Asn – Ser – Ser – Phe – Tyr – Arg – Leu – Arg – Phe – Glu – Leu – Arg – Tyr – Arg – Ala – Glu – Arg – Ser – Lys –

(g) – Gln – Ala – Leu – Thr – Thr – Asn – Lys – Asp – Asp – Asp – Asn – Ile – Leu – Phe – Arg – Asp – Ser – Ala –

(h) – His – Ser – Trp – Asn – Ser – Ser – Phe – Tyr – Arg – Leu – Arg – Phe – Glu – Leu – Arg – Tyr – Arg – Ala – Glu – Arg – Ser – Lys –

(i) – Pro – His – Ser – Trp – Asn – Ser – Ser – Phe – Tyr

- Arg - Leu - Arg - Phe - Glu - Leu - Arg - Tyr - Arg
- Ala - Glu - Arg - Ser - Lys -

(j) - Asp - Pro - His - Ser - Trp - Asn - Ser - Ser - Phe
- Tyr - Arg - Leu - Arg - Phe - Glu - Leu - Arg - Tyr
- Arg - Ala - Glu - Arg - Ser - Lys -

(k) - Gln - Asp - Pro - His - Ser - Trp - Asn - Ser - Ser
- Phe - Tyr - Arg - Leu - Arg - Phe - Glu - Leu - Arg
- Tyr - Arg - Ala - Glu - Arg - Ser - Lys -

(l) - Trp - Gln - Asp - Pro - His - Ser - Trp - Asn - Ser
- Ser - Phe - Tyr - Arg - Leu - Arg - Phe - Glu - Leu
- Arg - Tyr - Arg - Ala - Glu - Arg - Ser - Lys -

The peptide represented by the general formula (I) wherein A is a peptide segment formed by binding 5 or less amino acids has no ability to binding to IL-6, or its ability to binding to IL-6 is insufficient for the practical use. Further, it is not practical to synthesize a peptide segment being capable of binding to the desired IL-6 and formed by binding 51 or more amino acids.

The synthesis of the peptide of the general formula (I) can be carried out by the conventional method usually employed in peptide syntheses, for Example, a solid phase synthesis, or a liquid phase synthesis such as stepwise elongation, fragment condensation or the like. In view of the operation, a solid phase synthesis is convenient [see, for Example, Journal of the American Chemical Society, Vol. 85, pages 2149 to 2154 (1963); "Seikagaku Jikken Koza (Biochemical Experiment Lecture 1, Protein Chemistry IV, Chemical Modification and Peptide Synthesis)" edited by The Japanese Biochemical Society, published November 15, 1977 by Tokyo Kagaku Dojin Co., Ltd., pages 207 to 495; "Zoku-Seikagaku Jikken Koza (Biochemical Experiment Lecture Second Series 2, Protein Chemistry, the last volume)" edited by The Japanese Biochemical Society, published May 20, 1987 by Tokyo Kagaku Dojin Co., Ltd, pages 641 to 694; etc.].

The production of the peptide of the general formula (I) according to a solid phase synthesis is carried out by using a polymer such as styrene-divinylbenzene copolymer which is insoluble in a reaction solvent as a solid phase carrier. An amino acid or amino acid amide corresponding to the C-terminal of the desired peptide is bound to the solid phase carrier by utilizing α-COOH group or α-$CONH_2$ group thereof. Then, corresponding amino acids or peptide segments are bound to the amino acid or amino acid amide in order through peptide bonds toward the direction of the N-terminal of the desired peptide. In this case, usually, the amino acid or peptide segment to be bound is added after protection of any functional group of the C-terminal other than α-COOH group. In addition, usually, an amino acid, or amino acid amide or peptide segment on the solid phase carrier is subjected to a peptide bond formation reaction after removal of a protecting group only for the α-NH_2 group. Formation of peptide bonds are carried out by a known method such as a dehydration condensation method using carbodiimide or the like. The desired peptide can be obtained by forming a peptide chain corresponding to the desired peptide on a solid phase carrier, removing it from the solid phase carrier and removing any protecting group from any protected functional group and, if necessary, purifying the resulting peptide. In this case, removal of the peptide chain from the solid phase carrier and removal of the protecting group can be carried out by a known method and it is preferred that these operations are carried out at once by using hydrogen fluoride from the viewpoint of inhibition of a side reaction. Further, the purification of the resulting peptide can be efficiently carried out by reversed phase liquid chromatography.

Since the peptide of the general formula (I) is capable of binding to IL-6, it can inhibit binding of IL-6 to its receptor. Therefore, the production of autoantibody can be inhibited by administering the peptide of the

general formula (I) to a patient suffered from autoimmune diseases such as rheumatism and the like, wherein the production of an autoantibody caused by binding IL-6 to its receptor is accelerated.

A dosage to manifest an effective activity of the peptide of the general formula (I) is not more than 2 g/kg, preferably, not less than 1 μg/kg to not more than 200 mg/kg. As preferred dosage forms and routes of administration, for Example, there is a solution of the peptide of the general formula (I) dissolved in water or a physiologically acceptable salt solution such as physiological saline solution [e.g., a solution obtained by dissolving 1 mg of the peptide of the general formula (I) in 100 ml of 5% glucose solution or the like] by intravenous administration, subcutaneous administration, intraperitoneal administration and the like.

Further, the peptide of the general formula (I), or the above solution in water or a salt solution can be administered orally in the form of a capsule or liposome. It can also be administered percutaneously in the form of an oil. The peptide of the general formula (I) dose not manifest a remarkable acute toxicity at the above dosage.

Furthermore, the peptide of the general formula (I) is immobilized on a carrier and is used as an adsorbent of IL-6. One or more peptides of the general formula (I) can be used for the immobilization.

As the carrier to be used for immobilizing the peptide of the general formula (I), that having a hydrophilic surface and a reactive functional group such as amino group, carboxyl group, hydroxyl group or the like which can be utilized to form a covalent bonding with the peptide is preferable. Further, when the peptide is used as an adsorbent to adsorb IL-6 in a body fluid of a patient with an autoimmune disease, the above carrier is preferably insoluble to the body fluid and porous. As the porous carrier having a wide effective area to adsorb IL-6, a carrier having an exclusion limit protein molecular weight of about 10^6 to 10^9 or an average pore diameter of about 50 to 1000 nanometer can be preferably used. The carrier can be in any desired form such as particles, fibers, sheets, hollow fibers and the like. As these carrier, there are organic carriers, for Example, cellulose carriers such as CM-Cellulofine CH (exclusion limit protein molecular weight: about 3×10^6, sold by Seikagaku Kogyo Co., Ltd.) and the like, polyvinyl alcohol carriers such as TSK-gel CM-Toyopearl 650C (exclusion limit protein molecular weight: 5×10^6, manufactured by Toso Co., Ltd.), polyacrylamide carriers such as CM-Trisacryl M (exclusion limit protein molecular weight: 1×10^7, manufactured by Pharmacia-LKB, Sweden) and the like, agarose carriers such as Sepharose CL-4B (exclusion limit protein molecular weight: 2×10^7, manufactured by Pharmacia-LKB, Sweden) and the like; and inorganic carriers, for Example, porous glass such as CPG-10-1000 (exclusion limit protein molecular weight: 1×10^8, manufactured by Electro-nucleonics Co., U.S.A.) and the like.

Immobilization of the peptide of the general formula (I) on the carrier can be carried out according to a method generally employed in immobilization of a peptide or protein on a carrier. As methods for immobilization, for Example, there are a method comprising reacting carboxyl group contained in a carrier with N-hydroxysuccinimide to convert the carboxyl group into succinimidoxycarbonyl group and reacting this with the peptide of the general formula (I) at the amino group site (activated ester method); a method comprising condensing amino group or carboxyl group contained in a carrier with the peptide of the general formula (I) at the carboxyl group or amino group site in the presence of a condensation agent such as dicyclohexyl carbodiimide (condensation method); a method comprising crosslinking a carrier and the peptide of the general formula (I) with a compound having two or more functional groups such as glutaraldehyde (carrier crosslinking method), and the like. The adsorbent obtained by immobilizing the peptide of the general formula (I) on the carrier according to the activated ester method has a most highest adsorption capability of IL-6. Usually, the amount of the peptide of the general formula (I) immobilized on the carrier should be about 3×10^{-8} mole/g (carrier) or more so that the resulting adsorbent can adsorb a significant amount of IL-6, and about 1×10^{-7} to 2×10^{-6} mole/g (carrier) is preferable so that the peptide of the general formula (I) immobilized on the carrier can be efficiently utilized for adsorption of IL-6.

Removal of IL-6 can be carried out by contacting the adsorbent obtained by immobilizing the peptide of the general formula (I) on the carrier with a body fluid containing IL-6 such as blood, plasma, serum and the like to adsorb IL-6. For Example, the absorbent is used by packing it in a column. It is preferable that the column used for this purpose has inlet and outlet parts having the shape which can be easily connected to the blood circulation and is provided with filters of a material such as polyester between the inlet part and the adsorbent layer as well as between the outlet part and the adsorbent layer, respectively. Examples of the material for making the column include polyethylene, polypropylene, polycarbonate, polyester, polymethyl methacrylate and the like. Among these, polypropylene and polycarbonate are particularly suitable because the column packed with the adsorbent can be subjected to sterilization such as autoclave sterilization, Υ ray-sterilization and the like before use.

For Example, removal of IL-6 from the body fluid of a patient using a column packed with the above adsorbent can be carried out according to an extracorporeal blood circulation system. As the extracorporeal blood circulation system, for Example, there are following two systems:

(1) Blood from the blood vessel of a patient is transferred to a column packed with the adsorbent, followed by removal of IL-6 from blood by adsorption in the column. The blood thus treated by passing through the column is then circulated in the blood vessel of the patient;

(2) Blood from the blood vessel of a patient is firstly separated into the blood cell component and the plasma component and the separated plasma component is transferred to a column packed with the adsorbent. IL-6 is removed from the plasma component by adsorption in the column. Then, the plasma component thus treated by passing through the column is admixed with the above separated blood cell component, and the resulting mixture is circulated in the blood vessel of the patient.

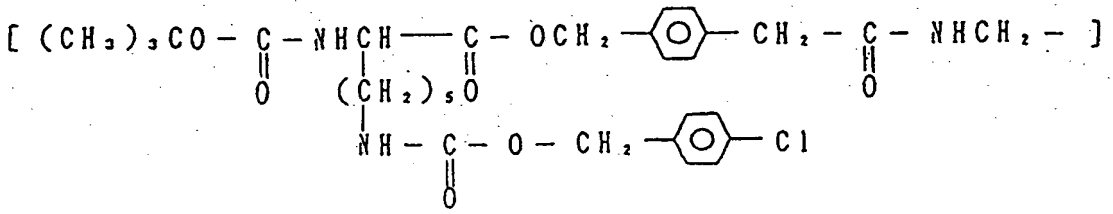
EMBODIMENT FOR WORKING THE INVENTION

The following Examples further illustrate the present invention in detail but are not to be construed to limit the scope thereof.

Example 1

A peptide of the formula: H-Thr-Ser-Leu-Pro-Gly-Ser-Val-Thr-Leu-Thr-Cys-Pro-Gly-Val-Glu-Pro-Glu-Asp-Lys-OH was synthesized by using an automatic peptide synthesizer [manufactured by Applied Biosystems Corp., U.S.A., Model 430A] according to solid phase synthetic method.

That is, 0.13 g of a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing 4-[N^{α}-(t-butoxycarbonyl)-N^{ϵ}-chlorbenzyloxycarbonyl)-L-lysyloxylmethyl]phenyacetamidomethyl group,



in a ratio of 0.78 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., PAM Lysine, t-Boc-L-Lys (Cl-Z)] was used for binding the corresponding L-aspartic acid, L-cysteine, glycine, L-glutamic acid, L-leucine, L-serine, L-proline, L-threonine and L-valine thereto in order toward the direction of the N-terminal of the desired peptide according to a series of operation as shown in Table 1. In the condensation reaction, the above amino acids were used as N-(t-butoxycarbonyl)-O^{β}-benzyl-L-aspartic acid anhydride, N-(t-butoxycarbonyl)-S-(p-methoxybenzyl)-L-cysteine anhydride, N-(t-butoxycarbonyl) glycine anhydride, N-(t-butoxycarbonyl)-O^{γ}-benzyl-L-glutamic acid anhydride, N-(t-butoxycarbonyl)-L-leucine anhydride, N-(t-butoxycarbonyl)-O-benzyl-L-serine anhydride, N-(t-butoxycarbonyl)-L-proline anhydride, N-(t-butoxycarbonyl)-O^{β}-benzyl-L-threonine anhydride and N-(t-butoxycarbonyl)-L-valine anhydride, respectively and their amounts were about five-fold molar amount based on the amount of the substrate. The condensation reaction was carried out at room temperature. The reaction time was varied depending on the kinds of amino acids to be condensed and ranged from 10 to 20 minutes.

Table 1

Operation	Solvent and/or reagent used	Time
1. Removal of t-butoxycarbonyl group	trifluoroacetic acid	5 minutes
2. Washing	N,N-dimethylformamide	40 seconds
3. Neutralization	N,N-dimethylformamide solution containing 20 % by volume of diisopropylethylamine	1 minute
4. Washing	N,N-dimethylformamide	40 seconds
5. Condensation reaction	N,N-dimethylformamide solution containing amino acid (10 to 25 ml)	10 to 20 minutes
6. Washing	dichloromethane	40 seconds

After completion of the reaction operation for all the amino acids, the resulting resin was washed on a glass filter with diethyl ether, dichloromethane and methanol in order and vacuum dried to produce 0.41 g of a dried resin. In a reaction vessel made of polytrifluoromonochloroethylene (manufactured by Peptide Kenkyusho Co., Ltd., HF-reaction apparatus, Type I), 0.41 g of the resulting dried resin was admixed with 0.6 ml of anisole and 0.1 ml of ethyl methyl sulfide and to the mixture was added 4 ml of hydrogen fluoride at -20 °C. The mixture was stirred at the same temperature for 30 minutes and then at 0 °C for 30 minutes. Hydrogen fluoride, anisole and ethyl methyl sulfide were removed from the resulting reaction mixture under reduced pressure and the residue was thoroughly washed on a glass filter with diethyl ether. The residue was extracted with a 2 N aqueous acetic acid solution and the extract was lyophilized to produce a crude product of peptide (0.2 g).

The resulting crude product of peptide was purified by preparative reversed phase high performance liquid chromatography [column: column (inner diameter: 10 mm, length: 300 mm) packed with octadecylated silica gel (grain size: 5 μm), manufactured by Chemco Co., Ltd., Develosil ODS; mobile phase: mixed solvent of acetonitrile containing 0.05% by volume of trifluoroacetic acid and water (the concentration of acetonitrile was gradually changed from 20% by volume to 35% by volume for 20 minutes.)] to obtain 50 mg of the desired purified product of peptide.

The resulting purified product of peptide was subjected to analytical reversed phase high performance liquid chromatography [column: column (inner diameter: 4 mm, length: 150 mm) packed with octadecylated silica gel (grain size: 5 μm), manufactured by Toso Co., Ltd., TSK gel ODS-80TM; mobile phase: mixed

solvent of acetonitrile containing 0.05% by volume of trifluoroacetic acid and water (the concentration of acetonitrile was gradually changed from 5% by volume to 50% by volume for 30 minutes); flow rate: 1 ml/minute; detection method: absorbance at wavelength of 210 nm] and the result showed a single sharp peak at 17.5 minutes. The molecular weight of the purified product obtained by mass spectrum according to fast atomic bombardment method (hereinafter abbreviated as FAB method) was 2046 (theoretical value: 2045.22). In addition, the purified product was hydrolyzed with hydrochloric acid and the resulting product was subjected to analysis of the amino acid composition. The results are as follows (figures in parentheses mean theoretical value):

lysine: 1.04 (1), aspartic acid: 2.09 (2), glutamic acid: 2.02 (2), proline: 3.10 (3), valine: 1.90 (2), glycine: 1.95 (2), cystine: 0.44 (0.5), threonine: 3.11 (3), leucine: 1.99 (2), serine: 1.98 (2).

Examples 2 to 96

According to the same manner as that described in Example 1, the solid phase synthesis of peptide and purification thereof were carried out to obtain the peptides shown in Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12 and Table 13. However, in Example 2, Example 5, Example 42 and Example 45, a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing 4-[N-(t-butoxycarbonyl)-O$^{\beta}$-benzyl-α-L-aspartyloxymethyl]phenylacetamidomethyl group in a ratio of 0.78 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., PAM Aspartic acid, t-Boc-L-Asp (OBzl)] was used as the resin for the solid phase. In Example 3, Example 11, Example 19, Example 27, Example 35, Example 43, Example 51, Example 59, Example 67, Example 75, Example 83 and Example 91, a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing 4-[N-(t-butoxycarbonyl)-O$^{\gamma}$-benzyl-α-L-glutamyloxymethyl]phenylacetamidomethyl group in a ratio of 0.78 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., PAM Glutamic acid, t-Boc-L-Glu (OBzl)] was used. In Example 4, Example 12, Example 20, Example 28, Example 36, Example 44, Example 52, Example 60, Example 68, Example 76, Example 84 and Example 92, a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing 4-[N-(t-butoxycarbonyl)-glycyloxymethyl]phenylacetamidomethyl group in a ratio of 0.78 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., PAM Glycine, t-Boc-Gly] was used. In Example 9, Example 10, Example 13, Example 17, Example 25, Example 33, Example 34, Example 37, Example 41, Example 49, Example 57, Example 58, Example 61, Example 65, Example 66, Example 69, Example 73, Example 74, Example 77, Example 81, Example 82, Example 85, Example 89, Example 90 and Example 93; a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing 4-[N$^{\alpha}$-(t-butoxycarbonyl)-N$^{\epsilon}$-chlorobenzyloxycarbonyl)-L-lysyloxymethyl]phenylacetamidomethyl group in a ratio of 0.78 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., PAM Lysine, t-Boc-L-Lys (Cl-Z)] was used. In Example 18, Example 21, Example 50 and Example 53, a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing 4-[N-(t-butoxylcarbonyl)-O-benzyl-L-seryloxymethyl]phenylacetamidomethyl group in a ratio of 0.78 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., PAM Serine, t-Boc-L-Ser] was used. In Example 26 and Example 29, a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing 4-[N-(t-butoxylcarbonyl)-L-alanyloxymethyl]-phenylacetamidomethyl group in a ratio of 0.76 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., PAM Alanine, t-Boc-L-Ala] was used. In Example 6, Example 7, Example 8, Example 14, Example 15, Example 16, Example 22, Example 23, Example 24, Example 30, Example 31, Example 32, Example 38, Example 39, Example 40, Example 46, Example 47, Example 48, Example 54, Example 55, Example 56, Example 62, Example 63, Example 64, Example 70, Example 71, Example 72, Example 78, Example 79, Example 80, Example 86, Example 87, Example 88, Example 94, Example 95 and Example 96, a granular resin of a styrene-divinylbenzene copolymer [molar ratio of styrene to divinylbenzene being 99 : 1] containing α-amino-p-methylbenzyl group in a ratio of 0.78 mmole/g (resin) [manufactured by Applied Biosystems Corp., U.S.A., p-Methyl BHA Resin] was used. And, in the condensation reaction, L-alanine, L-arginine, L-asparagine, L-glutamine, L-histidine, L-isoleucine, L-lysine, L-phenylalanine, L-tryptophan, L-tyrosine, 12-aminododecanoic acid and 18-aminooctadecanoic acid were used as N-(t-butoxycarbonyl)-L-alanine anhydride, N-(t-butoxycarbonyl)-(2,4,6-trimethyl) benzene sulfonyl-L-arginine hydroxybenzotriazyl ester, N-(t-butoxycarbonyl)-L-aspargine hydroxybenzotriazyl ester, N-(t-butoxycarbonyl)-L-glutamine hydroxybenzotriazyl ester, N$^{\alpha}$-(t-butoxycarbonyl)-N^{im}-dinitrophenyl-L-histidine hydroxybenzotriazyl ester, N-(t-butoxycarbonyl)-L-isoleucine anhydride, N$^{\alpha}$-(t-butoxycarbonyl)-N$^{\epsilon}$-2-chlorobenzyloxycarbonyl-L-lysine anhydride, N-(t-

butoxycarbonyl)-L-phenylalanine anhydride, N-(t-butoxycarbonyl)-N^{im}-formyl-L-tryptophan anhydride, N-(t-butoxycarbonyl)-O-(p-bromo) benzyloxycarbonyl-L-tyrosine anhydride, 12-(t-butoxycarbonylamino) dodecanoic acid anhydride and 18-(t-butoxycarbonylamino) octadecanoic acid anhydride, respectively.

When the resulting purified products were subjected to analytical reversed phase high performance liquid chromatography [column: column (inner diameter: 4 mm, length: 150 mm) packed with octadecylated silica gel (grain size: 5 μm), manufactured by Toso Co., Ltd., TSK gel ODS-80TM; mobile phase: mixed solvent of acetonitrile containing 0.05% by volume of trifluoroacetic acid and water (the concentration of acetonitrile was gradually changed from 5% by volume to 50% by volume for 30 minutes); flow rate: 1 ml/minute; detection method: absorbance at wavelength of 210 nm], they showed a single sharp peak. The molecular weight of the purified products obtained by mass spectrum according to FAB method and the values of amino acid composition analysis of the products obtained by hydrolysis with hydrochloric acid are shown in Table 14, respectively.

Table 2

	A : −Thr−Ser−Leu−Pro−Gly−Asp−Ser−Val−Thr−Leu−Thr−Cys−Pro−Gly−Val−Glu−Pro−Glu−Asp−		
Example	X	Y	Z
2	$\leftarrow Lys \rightarrow_2$	−	OH
3	$-NH\leftarrow CH_2 \rightarrow_{11} CO-$	$\leftarrow Glu \rightarrow_5$	OH
4	$-NH\leftarrow CH_2 \rightarrow_{17} CO-$	−Gly−	OH
5	−	−	OH
6	−Lys−	−Asp−	NH_2
7	$\leftarrow Glu \rightarrow_3$	−Lys−Gly−	NH_2
8	$\leftarrow Asp \rightarrow_5$	−Ala−Ala−Gly−	NH_2

Table 3

	A : −Gly−Thr−Val−His−Leu−Leu−Val−Asp−Val−Pro−Pro−Glu−Glu−Pro−Gln−Leu−Ser−Cys−Phe−Arg−Lys−		
Example	X	Y	Z
9	−	−Lys−	OH
10	$\leftarrow Lys \rightarrow_2$	−	OH
11	$-NH\leftarrow CH_2 \rightarrow_{11} CO-$	$\leftarrow Glu \rightarrow_5$	OH
12	$-NH\leftarrow CH_2 \rightarrow_{17} CO-$	−Gly−	OH
13	−	−	OH
14	−Lys−	−Asp−	NH_2
15	$\leftarrow Glu \rightarrow_3$	−Lys−Gly−	NH_2
16	$\leftarrow Asp \rightarrow_5$	−Ala−Ala−Gly−	NH_2

Table 4

A : -Arg-Lys-Phe-Gln-Asn-Ser-Pro-Ala-Glu-Asp-Phe-Gln-Glu-Pro-Cys-Gln-Tyr-Ser-Gln-Glu-Ser-

Example	X	Y	Z
17	-	-Lys-	OH
18	$\leftarrow$Lys$\rightarrow_2$	-	OH
19	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
20	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
21	-	-	OH
22	-Lys-	-Asp-	NH$_2$
23	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH$_2$
24	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH$_2$

Table 5

A : -Gln-Ala-Leu-Thr-Thr-Asn-Lys-Asp-Asp-Asp-Asn-Ile-Leu-Phe-Arg-Asp-Ser-Ala-

Example	X	Y	Z
25	-	-Lys-	OH
26	$\leftarrow$Lys$\rightarrow_2$	-	OH
27	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
28	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
29	-	-	OH
30	-Lys-	-Asp-	NH$_2$
31	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH$_2$
32	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH$_2$

Table 6

A : -Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-

Example	X	Y	Z
33	-	-Lys-	OH
34	$\leftarrow$Lys$\rightarrow_2$	-	OH
35	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
36	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
37	-	-	OH
38	-Lys-	-Asp-	NH_2
39	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH_2
40	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH_2

Table 7

A : -Ser-Thr-Pro-Ser-Leu-Thr-Thr-Lys-Ala-Val-Leu-Leu-Val-Arg-Lys-Phe-Glu-Asn-Ser-Pro-Ala-Glu-Asp-

Example	X	Y	Z
41	-	-Lys-	OH
42	$\leftarrow$Lys$\rightarrow_2$	-	OH
43	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
44	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
45	-	-	OH
46	-Lys-	-Asp-	NH_2
47	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH_2
48	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH_2

Table 8

Example	X	Y	Z
	A : -Asn-Pro-Arg-Trp-Leu-Ser-Val-Thr-Trp-Gln-Asp-Pro-His-Ser-		
49	-	-Lys-	OH
50	$\leftarrow$Lys$\rightarrow_2$	-	OH
51	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
52	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
53	-	-	OH
54	-Lys-	-Asp-	NH_2
55	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH_2
56	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH_2

Table 9

Example	X	Y	Z
	A : -His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-		
57	-	-Lys-	OH
58	$\leftarrow$Lys$\rightarrow_2$	-	OH
59	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
60	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
61	-	-	OH
62	-Lys-	-Asp-	NH_2
63	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH_2
64	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH_2

Table 10

A : -Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-

Example	X	Y	Z
65	-	-Lys-	OH
66	$\leftarrow$Lys$\rightarrow_2$	-	OH
67	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
68	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
69	-	-	OH
70	-Lys-	-Asp-	NH_2
71	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH_2
72	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH_2

Table 11

A : -Asp-Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-

Example	X	Y	Z
73	-	-Lys-	OH
74	$\leftarrow$Lys$\rightarrow_2$	-	OH
75	-NH$\leftarrow$CH$_2\rightarrow_{11}$CO-	$\leftarrow$Glu$\rightarrow_5$	OH
76	-NH$\leftarrow$CH$_2\rightarrow_{17}$CO-	-Gly-	OH
77	-	-	OH
78	-Lys-	-Asp-	NH_2
79	$\leftarrow$Glu$\rightarrow_3$	-Lys-Gly-	NH_2
80	$\leftarrow$Asp$\rightarrow_5$	-Ala-Ala-Gly-	NH_2

Table 12

A : -Gln-Asp-Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-

Example	X	Y	Z
81	-	-Lys-	OH
82	$(Lys)_2$	-	OH
83	$-NH(CH_2)_{11}CO-$	$(Glu)_5$	OH
84	$-NH(CH_2)_{17}CO-$	-Gly-	OH
85		-	OH
86	-Lys-	-Asp-	NH_2
87	$(Glu)_3$	-Lys-Gly-	NH_2
88	$(Asp)_5$	-Ala-Ala-Gly-	NH_2

Table 13

A : -Trp-Gln-Asp-Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-

Example	X	Y	Z
89	-	-Lys-	OH
90	$(Lys)_2$	-	OH
91	$-NH(CH_2)_{11}CO-$	$(Glu)_5$	OH
92	$-NH(CH_2)_{17}CO-$	-Gly-	OH
93	-	-	OH
94	-Lys-	-Asp-	NH_2
95	$(Glu)_3$	-Lys-Gly-	NH_2
96	$(Asp)_5$	-Ala-Ala-Gly-	NH_2

Table 14

Example	2	3	4	5	6
Molecular weight by FAB method mass spectrum	2172 (2173.39)	2760 (2759.93)	2255 (2255.56)	1916 (1917.05)	2158 (2159.31)
amino acid composition analysis					
Alanine	–	–	–	–	–
Arginine	–	–	–	–	–
Asparagine	–	–	–	–	–
Aspartic acid	2.08 (2)	2.07 (2)	2.05 (2)	2.06 (2)	3.10 (3)
Cystine	0.43 (0.5)	0.45 (0.5)	0.42 (0.5)	0.44 (0.5)	0.43 (0.5)
Glutamine	–	–	–	–	–
Glutamic acid	2.03 (2)	7.22 (7)	2.04 (2)	2.02 (2)	2.03 (2)
Glycine	1.96 (2)	1.96 (2)	2.92 (3)	1.97 (2)	1.98 (2)
Histidine	–	–	–	–	–
Isoleucine	–	–	–	–	–
Leucine	1.98 (2)	1.97 (2)	1.98 (2)	1.97 (2)	1.98 (2)
Lysine	2.07 (2)	–	–	–	1.02 (1)
Phenylalanine	–	–	–	–	–
Proline	3.12 (3)	3.11 (3)	3.10 (3)	3.10 (3)	3.09 (3)
Serine	1.97 (2)	1.96 (2)	1.96 (2)	1.98 (2)	1.97 (2)
Threonine	3.12 (3)	3.11 (3)	3.11 (3)	3.10 (3)	3.12 (3)
Tyrosine	–	–	–	–	–
Valine	1.92 (2)	1.93 (2)	1.94 (2)	1.96 (2)	1.94 (2)
$H_2N(CH_2)_{11}COOH$	–	1.01 (1)	–	–	–
$H_2N(CH_2)_{12}COOH$	–	–	1.02 (1)	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	7	8	9	10	11
Molecular weight by FAB method mass spectrum	2489 (2489.616)	2690 (2690.70)	2491 (2492.88)	2620 (2621.06)	3207 (3207.59)
amino acid composition analysis					
Alanine	–	1.98 (2)	–	–	–
Arginine	–	–	0.98 (1)	0.96 (1)	0.97 (1)
Asparagine	–	–	–	–	–
Aspartic acid	2.06 (2)	7.21 (7)	1.02 (1)	1.01 (1)	1.02 (1)
Cystine	0.42 (0.5)	0.43 (0.5)	0.40 (0.5)	0.42 (0.5)	0.43 (0.5)
Glutamine	–	–	0.90 (1)	0.88 (1)	0.89 (1)
Glutamic acid	5.10 (5)	2.03 (2)	2.04 (2)	2.03 (2)	7.20 (7)
Glycine	2.95 (3)	2.98 (3)	0.99 (1)	0.98 (1)	0.99 (1)
Histidine	–	–	0.98 (1)	0.98 (1)	0.97 (1)
Isoleucine	–	–	–	–	–
Leucine	1.97 (2)	1.98 (2)	2.97 (3)	2.96 (3)	2.95 (3)
Lysine	1.01 (1)	–	2.03 (2)	2.05 (3)	1.02 (1)
Phenylalanine	–	–	1.01 (1)	1.02 (1)	1.02 (1)
Proline	3.08 (3)	3.09 (3)	3.06 (3)	3.07 (3)	3.10 (3)
Serine	1.98 (2)	1.97 (2)	0.98 (1)	0.99 (1)	0.97 (1)
Threonine	3.09 (3)	3.09 (3)	1.03 (1)	1.02 (1)	1.03 (1)
Tyrosine	–	–	–	–	–
Valine	1.98 (2)	1.98 (2)	2.90 (3)	2.92 (3)	2.97 (3)
$H_2N(CH_2)_{11}COOH$	–	–	–	–	0.99 (1)
$H_2N(CH_2)_{12}COOH$	–	–	–	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	12	13	14	15	16
Molecular weight by FAB method mass spectrum	2702 (2703.23)	2364 (2364.71)	2606 (2606.97)	2936 (2937.28)	3138 (3138.36)
amino acid composition analysis					
Alanine	–	–	–	–	1.97 (2)
Arginine	0.96 (1)	0.98 (1)	0.98 (1)	0.96 (1)	0.97 (1)
Asparagine	–	–	–	–	–
Aspartic acid	1.03 (1)	1.01 (1)	2.02 (2)	1.01 (1)	6.17 (6)
Cystine	0.43 (0.5)	0.40 (0.5)	0.44 (0.5)	0.40 (0.5)	0.42 (0.5)
Glutamine	0.90 (1)	0.90 (1)	0.91 (1)	0.89 (1)	0.91 (1)
Glutamic acid	2.02 (2)	2.04 (2)	2.03 (2)	5.09 (5)	2.03 (2)
Glycine	1.99 (2)	0.98 (1)	0.99 (1)	1.98 (2)	1.96 (2)
Histidine	0.98 (1)	0.98 (1)	0.99 (1)	0.99 (1)	0.97 (1)
Isoleucine	–	–	–	–	–
Leucine	2.96 (3)	2.94 (3)	2.92 (3)	2.95 (3)	2.96 (3)
Lysine	1.01 (1)	1.01 (1)	2.02 (2)	2.03 (2)	1.02 (1)
Phenylalanine	1.02 (1)	1.02 (1)	1.02 (1)	1.01 (1)	1.03 (1)
Proline	3.08 (3)	3.12 (3)	3.10 (3)	3.08 (3)	3.10 (3)
Serine	0.98 (1)	0.97 (1)	0.97 (1)	0.99 (1)	0.98 (1)
Threonine	1.01 (1)	1.02 (1)	1.01 (1)	1.03 (1)	1.03 (1)
Tyrosine	–	–	–	–	–
Valine	2.92 (3)	2.95 (3)	2.95 (3)	2.96 (3)	2.96 (3)
H,N⟨CH,⟩,,COOH	–	–	–	–	–
H,N⟨CH,⟩,,COOH	1.00 (1)	–	–	–	–

Note: Figures in parenthesis are theoretical value.

EP 0 426 857 A1

Table 14 (continued)

Example	17	18	19	20	21
Molecular weight by FAB method mass spectrum	2646 (2646.80)	2774 (2774.97)	3360 (3361.50)	2856 (2857.14)	2518 (2518.63)
amino acid composition analysis					
Alanine	1.01 (1)	1.02 (1)	1.01 (1)	1.01 (1)	1.00 (1)
Arginine	0.96 (1)	0.95 (1)	0.94 (1)	0.95 (1)	0.94 (1)
Asparagine	0.92 (1)	0.90 (1)	0.89 (1)	0.90 (1)	0.90 (1)
Aspartic acid	1.01 (1)	1.00 (1)	1.02 (1)	1.01 (1)	1.00 (1)
Cystine	0.44 (0.5)	0.44 (0.5)	0.43 (0.5)	0.42 (0.5)	0.44 (0.5)
Glutamine	3.75 (4)	3.79 (4)	3.72 (4)	3.80 (4)	3.82 (4)
Glutamic acid	3.01 (3)	3.03 (3)	8.21 (8)	3.02 (3)	3.05 (3)
Glycine	–	–	–	1.00 (1)	–
Histidine	–	–	–	–	–
Isoleucine	–	–	–	–	–
Leucine	–	–	–	–	–
Lysine	2.02 (2)	3.04 (3)	1.01 (1)	1.02 (1)	1.01 (1)
Phenylalanine	2.02 (2)	2.03 (2)	2.03 (2)	2.02 (2)	2.04 (2)
Proline	2.05 (2)	2.06 (2)	2.05 (2)	2.03 (2)	2.03 (2)
Serine	2.97 (3)	2.97 (3)	2.95 (3)	2.96 (3)	2.95 (3)
Threonine	–	–	–	–	–
Tyrosine	0.98 (1)	0.97 (1)	0.97 (1)	0.98 (1)	0.99 (1)
Valine	–	–	–	–	–
H,N(CH,),,COOH	–	–	1.01 (1)	–	–
H,N(CH,),,COOH	–	–	–	0.99 (1)	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	22	23	24	25	26
Molecular weight by FAB method mass spectrum	2760 (2760.89)	3090 (3091.19)	3291 (3292.28)	2165 (2165.32)	2293 (2293.50)
amino acid composition analysis					
Alanine	0.99 (1)	1.01 (1)	2.95 (3)	2.02 (2)	2.03 (2)
Arginine	0.92 (1)	0.95 (1)	0.95 (1)	0.96 (1)	0.96 (1)
Asparagine	0.89 (1)	0.91 (1)	0.92 (1)	1.87 (2)	1.85 (2)
Aspartic acid	1.98 (2)	1.01 (1)	6.18 (6)	3.99 (5)	3.95 (4)
Cystine	0.40 (0.5)	0.44 (0.5)	0.44 (0.5)	–	–
Glutamine	3.74 (4)	3.85 (4)	3.84 (4)	0.95 (1)	0.92 (1)
Glutamic acid	3.01 (3)	6.10 (6)	3.06 (3)	–	–
Glycine	–	0.99 (1)	0.98 (1)	–	–
Histidine	–	–	–	–	–
Isoleucine	–	–	–	1.01 (1)	0.99 (1)
Leucine	–	–	–	1.98 (2)	1.97 (2)
Lysine	2.01 (2)	2.02 (2)	1.01 (1)	2.04 (2)	3.06 (3)
Phenylalanine	2.02 (2)	2.03 (2)	2.03 (2)	1.01 (1)	1.00 (1)
Proline	2.01 (2)	2.03 (2)	2.02 (2)	–	–
Serine	2.90 (3)	2.93 (3)	2.97 (3)	0.98 (1)	0.97 (1)
Threonine	–	–	–	2.03 (2)	2.02 (2)
Tyrosine	0.97 (1)	0.99 (1)	0.99 (1)	–	–
Valine	–	–	–	–	–
$H_2N(CH_2)_{10}COOH$	–	–	–	–	–
$H_2N(CH_2)_{11}COOH$	–	–	–	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	27	28	29	30	31
Molecular weight by FAB method mass spectrum	2880 (2880.03)	2376 (2375.67)	2037 (2037.15)	2279 (2279.41)	2610 (2609.72)
amino acid composition analysis					
Alanine	2.03 (2)	2.02 (2)	2.03 (2)	2.02 (2)	2.02 (2)
Arginine	0.97 (1)	0.97 (1)	0.96 (1)	0.96 (1)	0.94 (1)
Asparagine	1.86 (2)	1.85 (2)	1.82 (2)	1.84 (2)	1.84 (1)
Aspartic acid	3.94 (4)	3.96 (4)	3.95 (4)	4.90 (5)	3.92 (4)
Cystine	–	–	–	–	–
Glutamine	0.91 (1)	0.92 (1)	0.90 (1)	0.92 (1)	0.91 (1)
Glutamic acid	5.06 (5)	–	–	–	3.05 (5)
Glycine	–	0.99 (1)	–	–	0.99 (1)
Histidine	–	–	–	–	–
Isoleucine	0.98 (1)	0.99 (1)	1.01 (1)	1.02 (1)	1.02 (1)
Leucine	1.98 (2)	1.97 (2)	1.96 (2)	1.95 (2)	1.97 (2)
Lysine	1.01 (1)	1.01 (1)	1.01 (1)	2.02 (2)	2.01 (2)
Phenylalanine	1.03 (1)	1.01 (1)	1.03 (1)	1.01 (1)	1.02 (1)
Proline	–	–	–	–	–
Serine	0.96 (1)	0.98 (1)	0.99 (1)	0.98 (1)	0.98 (1)
Threonine	2.03 (2)	2.02 (2)	2.02 (2)	2.01 (2)	2.01 (2)
Tyrosine	–	–	–	–	–
Valine	–	–	–	–	–
H,N(CH,)$_4$CH(NH$_2$)COOH	0.98 (1)	–	–	–	–
H,N(CH,)$_3$CH(NH$_2$)COOH	–	0.99 (1)	–	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	32
Molecular weight by FAB method mass spectrum	2811 (2810.80)
amino acid composition analysis	
Alanine	4.07 (4)
Arginine	0.97 (1)
Asparagine	1.83 (2)
Aspartic acid	8.89 (9)
Cystine	–
Glutamine	0.91 (1)
Glutamic acid	–
Glycine	0.98 (1)
Histidine	–
Isoleucine	1.01 (1)
Leucine	1.96 (2)
Lysine	1.01 (1)
Phenylalanine	1.01 (1)
Proline	–
Serine	0.97 (1)
Threonine	2.03 (2)
Tyrosine	–
Valine	–
H,N⟨CH,⟩₁₁COOH	–
H,N⟨CH,⟩₁₂COOH	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	33	34	35	36	37
Molecular weight by FAB method mass spectrum	2793 (2793.15)	2921 (2921.32)	3508 (3507.72)	3003 (3003.30)	2665 (2664.97)
amino acid composition analysis					
Alanine	1.01 (1)	0.98 (1)	0.99 (1)	0.99 (1)	0.98 (1)
Arginine	4.83 (5)	4.89 (5)	4.86 (5)	4.90 (5)	4.86 (5)
Asparagine	0.91 (1)	0.93 (1)	0.92 (1)	0.90 (1)	0.91 (1)
Aspartic acid	–	–	–	–	–
Cystine	–	–	–	–	–
Glutamine	–	–	–	–	–
Glutamic acid	2.02 (2)	2.03 (2)	7.22 (7)	2.04 (2)	2.02 (2)
Glycine	–	–	–	0.97 (1)	–
Histidine	–	–	–	–	–
Isoleucine	–	–	–	–	–
Leucine	1.99 (2)	1.98 (2)	1.97 (2)	1.98 (2)	1.97 (2)
Lysine	2.07 (2)	3.11 (3)	1.02 (1)	1.02 (1)	1.03 (1)
Phenylalanine	2.02 (2)	2.03 (2)	2.03 (2)	2.01 (2)	2.01 (2)
Proline	–	–	–	–	–
Serine	2.99 (3)	2.97 (3)	2.96 (3)	2.96 (3)	2.98 (3)
Threonine	–	–	–	–	–
Tyrosine	1.97 (2)	1.95 (2)	1.94 (2)	1.95 (2)	1.97 (2)
Tryptophan	1.01 (1)	1.02 (1)	1.02 (1)	1.01 (1)	1.02 (1)
$H_2N(CH_2)_{11}COOH$	–	–	1.01 (1)	–	–
$H_2N(CH_2)_{10}COOH$	–	–	–	1.02 (1)	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	38	39	40	41	42
Molecular weight by FAB method mass spectrum	2907 (2907.25)	3237 (3236.56)	3439 (3438.64)	2631 (2630.99)	2759 (2759.16)
amino acid composition analysis					
Alanine	0.97 (1)	0.99 (1)	2.97 (3)	1.96 (2)	1.97 (2)
Arginine	4.91 (5)	4.88 (5)	4.89 (5)	0.98 (1)	0.96 (1)
Asparagine	0.92 (1)	0.93 (1)	0.90 (1)	0.92 (1)	0.90 (1)
Aspartic acid	1.02 (1)	–	5.13 (5)	1.02 (1)	1.01 (1)
Cystine	–	–	–	–	–
Glutamine	–	–	–	0.90 (1)	0.88 (1)
Glutamic acid	2.03 (2)	5.10 (5)	2.03 (2)	1.04 (1)	1.04 (1)
Glycine	–	0.98 (1)	0.98 (1)	–	–
Histidine	–	–	–	–	–
Isoleucine	1.01 (1)	1.02 (1)	1.02 (1)	–	–
Leucine	1.98 (2)	1.97 (2)	1.98 (2)	2.97 (3)	2.96 (3)
Lysine	2.05 (2)	2.04 (2)	1.01 (1)	3.01 (3)	4.03 (4)
Phenylalanine	2.01 (2)	2.02 (2)	2.03 (2)	1.01 (1)	1.02 (1)
Proline	–	–	–	2.02 (2)	2.04 (2)
Serine	2.97 (3)	2.98 (3)	2.97 (3)	2.98 (3)	2.99 (3)
Threonine	–	–	–	3.05 (3)	3.09 (3)
Tyrosine	1.94 (2)	1.96 (2)	1.96 (2)	–	–
Valine	–	–	–	1.99 (2)	1.97 (2)
$H_2N(CH_2)_{11}COOH$	–	–	–	–	–
$H_2N(CH_2)_{10}COOH$	–	–	–	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	43	44	45	46
Molecular weight by FAB method mass spectrum	3345 (3345.57)	2841 (2841.14)	2503 (2502.52)	2744 (2745.10)
amino acid composition analysis				
Alanine	1.98 (2)	1.98 (2)	1.97 (2)	1.96 (2)
Arginine	0.97 (1)	0.96 (1)	0.98 (1)	0.98 (1)
Asparagine	0.91 (1)	0.90 (1)	0.91 (1)	0.93 (1)
Aspartic acid	1.02 (1)	1.03 (1)	1.01 (1)	2.02 (2)
Cystine	–	–	–	–
Glutamine	0.89 (1)	0.90 (1)	0.90 (1)	0.91 (1)
Glutamic acid	6.12 (6)	1.03 (1)	1.03 (1)	1.02 (1)
Glycine	–	0.99 (1)	–	–
Histidine	–	–	–	–
Isoleucine	–	–	–	–
Leucine	2.95 (3)	2.96 (3)	2.94 (3)	2.92 (3)
Lysine	2.01 (2)	2.03 (2)	2.02 (2)	3.02 (3)
Phenylalanine	1.02 (1)	1.02 (1)	1.02 (1)	1.02 (1)
Proline	2.01 (2)	2.03 (2)	2.03 (2)	2.02 (2)
Serine	2.97 (3)	2.95 (3)	2.96 (3)	2.97 (3)
Threonine	3.03 (3)	3.03 (3)	3.06 (3)	3.05 (3)
Tyrosine	–	–	–	–
Valine	1.98 (2)	1.98 (2)	2.00 (2)	1.99 (2)
$H_2N(CH_2)_4COOH$	0.99 (1)	–	–	–
$H_2N(CH_2)_3COOH$	–	1.00 (1)	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	47	48	49	50	51
Molecular weight by FAB method mass spectrum	3074 (3074.41)	3276 (3276.49)	1851 (1851.03)	1979 (1979.20)	2566 (2565.61)
amino acid composition analysis					
Alanine	1.98 (2)	3.92 (4)	–	–	–
Arginine	0.96 (1)	0.97 (1)	0.96 (1)	0.95 (1)	0.94 (1)
Asparagine	0.90 (1)	0.92 (1)	0.91 (1)	1.90 (1)	0.89 (1)
Aspartic acid	1.01 (1)	6.17 (6)	1.01 (1)	1.00 (1)	1.02 (1)
Cystine	–	–	–	–	–
Glutamine	0.89 (1)	0.91 (1)	0.90 (1)	0.92 (1)	0.94 (1)
Glutamic acid	4.08 (4)	1.02 (1)	–	–	5.21 (5)
Glycine	0.98 (1)	0.99 (1)	–	–	–
Histidine	–	–	0.98 (1)	0.98 (1)	0.99 (1)
Isoleucine	–	–	–	–	–
Leucine	2.95 (3)	2.96 (3)	–	–	–
Lysine	3.04 (3)	2.03 (2)	1.01 (1)	2.03 (2)	–
Phenylalanine	1.01 (1)	1.03 (1)	–	–	–
Proline	2.03 (2)	2.03 (2)	2.05 (2)	2.06 (2)	2.05 (2)
Serine	2.96 (3)	2.98 (3)	1.97 (2)	1.98 (2)	1.95 (2)
Threonine	3.09 (3)	3.05 (3)	–	–	–
Tryptophan	–	–	2.01 (2)	2.03 (2)	2.03 (2)
Valine	1.98 (2)	1.99 (2)	0.97 (1)	0.98 (1)	0.98 (1)
$H_2N(CH_2)_{11}COOH$	–	–	–	–	1.01 (1)
$H_2N(CH_2)_{12}COOH$	–	–	–	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	52	53	54	55	56
Molecular weight by FAB method mass spectrum	2061 (2061.18)	1723 (1722.86)	1965 (1965.14)	2294 (2294.44)	2497 (2496.52)
amino acid composition analysis					
Alanine	–	–	–	–	2.01 (2)
Arginine	0.95 (1)	0.94 (1)	0.92 (1)	0.95 (1)	0.95 (1)
Asparagine	0.90 (1)	0.90 (1)	0.89 (1)	0.91 (1)	0.92 (1)
Aspartic acid	1.01 (1)	1.00 (1)	1.98 (2)	1.01 (1)	6.18 (6)
Cystine	–	–	–	–	–
Glutamine	0.95 (1)	0.96 (1)	0.95 (1)	0.96 (1)	0.96 (1)
Glutamic acid	–	–	–	3.10 (3)	–
Glycine	1.00 (1)	–	–	0.99 (1)	0.98 (1)
Histidine	0.99 (1)	0.99 (1)	0.98 (1)	0.97 (1)	0.99 (1)
Isoleucine	–	–	–	–	–
Leucine	–	–	–	–	–
Lysine	–	–	1.00 (1)	1.02 (1)	–
Proline	2.03 (2)	2.03 (2)	2.01 (2)	2.03 (2)	2.02 (2)
Serine	1.97 (2)	1.95 (2)	1.97 (2)	1.96 (2)	1.97 (2)
Threonine	–	–	–	–	–
Tryptophan	2.03 (2)	2.02 (2)	2.01 (2)	2.01 (2)	2.03 (2)
Valine	0.98 (1)	0.99 (1)	0.99 (1)	0.97 (1)	0.99 (1)
$H_2N(CH_2)_{11}COOH$	–	–	–	–	–
$H_2N(CH_2)_{10}COOH$	0.99 (1)	–	–	–	–

Note: Figures in parenthesis are theoretical value.

EP 0 426 857 A1

Table 14 (continued)

Example	57	58	59	60	61
Molecular weight by FAB method mass spectrum	3017 (3017.36)	3146 (3145.53)	3732 (3731.94)	3228 (3227.51)	2890 (2889.19)
amino acid composition analysis					
Alanine	0.98 (1)	1.00 (1)	0.99 (1)	0.99 (1)	0.97 (1)
Arginine	4.79 (5)	4.82 (5)	4.77 (5)	4.75 (5)	4.77 (5)
Asparagine	0.90 (1)	0.91 (1)	0.90 (1)	0.88 (1)	0.87 (1)
Glutamic acid	2.03 (2)	2.02 (2)	7.12 (7)	2.05 (2)	2.03 (2)
Glycine	–	–	–	0.99 (1)	–
Histidine	0.99 (1)	0.99 (1)	1.00 (1)	0.97 (1)	0.98 (1)
Leucine	1.98 (2)	1.97 (2)	1.96 (2)	1.97 (2)	1.97 (2)
Lysine	2.03 (2)	3.04 (3)	1.02 (1)	1.01 (1)	1.00 (1)
Phenylalanine	2.03 (2)	2.01 (2)	2.04 (2)	2.02 (2)	2.03 (2)
Serine	3.94 (4)	3.92 (4)	3.93 (4)	3.91 (4)	3.92 (4)
Tyrosine	1.97 (2)	1.96 (2)	1.98 (2)	1.97 (2)	1.97 (2)
Tryptophan	1.02 (1)	1.00 (1)	1.03 (1)	1.02 (1)	1.03 (1)
$H_2N(CH_2)_{11}COOH$	–	–	0.97 (1)	–	–
$H_2N(CH_2)_{10}COOH$	–	–	–	0.96 (1)	–

Note: Figures in parenthesis are theoretical value.

EP 0 426 857 A1

Table 14 (continued)

Example	6 2	6 3	6 4	6 5	6 6
Molecular weight by FAB method mass spectrum	3131 (3131.47)	3461 (3460.77)	3663 (3662.85)	3114 (3114.47)	3243 (3242.64)
amino acid composition analysis					
Alanine	1.00 (1)	0.99 (1)	2.97 (3)	1.00 (1)	0.98 (1)
Arginine	4.78 (5)	4.80 (5)	4.80 (5)	4.77 (5)	4.75 (5)
Asparagine	0.92 (1)	0.91 (1)	0.93 (1)	0.89 (1)	0.91 (1)
Aspartic acid	1.01 (1)	–	5.18 (5)	–	–
Glutamic acid	2.03 (2)	5.08 (5)	2.03 (2)	2.02 (2)	2.03 (2)
Glycine	–	0.99 (1)	1.00 (1)	–	–
Histidine	0.98 (1)	0.97 (1)	0.99 (1)	1.00 (1)	0.98 (1)
Leucine	1.96 (2)	1.96 (2)	1.97 (2)	1.97 (2)	1.96 (2)
Lysine	2.02 (2)	2.01 (2)	1.03 (1)	2.03 (2)	3.05 (3)
Proline	–	–	–	1.01 (1)	1.02 (1)
Phenylalanine	2.03 (2)	2.04 (2)	2.04 (2)	2.02 (2)	2.03 (2)
Serine	3.92 (4)	3.92 (4)	3.92 (4)	3.93 (4)	3.91 (4)
Tyrosine	1.98 (2)	1.97 (2)	1.97 (2)	1.96 (2)	1.98 (2)
Tryptophan	1.02 (1)	1.03 (1)	1.02 (1)	1.01 (1)	1.03 (1)

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	67	68	69	70	71
Molecular weight by FAB method mass spectrum	3830 (3829.05)	3324 (3324.62)	2986 (2986.30)	3229 (3228.58)	3559 (3557.88)
amino acid composition analysis					
Alanine	1.00 (1)	0.97 (1)	1.01 (1)	0.98 (1)	0.98 (1)
Arginine	4.83 (5)	4.70 (5)	4.73 (5)	4.75 (5)	4.72 (5)
Asparagine	0.92 (1)	0.92 (1)	0.91 (1)	0.92 (1)	0.90 (1)
Aspartic acid	–	–	–	1.02 (1)	–
Glutamic acid	7.20 (7)	2.04 (2)	2.03 (2)	2.04 (2)	5.12 (5)
Glycine	–	0.99 (1)	–	–	1.00 (1)
Histidine	0.97 (1)	0.98 (1)	0.99 (1)	0.98 (1)	0.98 (1)
Leucine	1.97 (2)	1.96 (2)	1.96 (2)	1.97 (2)	1.96 (2)
Lysine	1.00 (1)	1.01 (1)	1.01 (1)	2.03 (2)	2.02 (2)
Proline	1.03 (1)	1.02 (1)	1.02 (1)	1.03 (1)	1.02 (1)
Phenylalanine	2.04 (2)	2.03 (2)	2.03 (2)	2.02 (2)	2.03 (2)
Serine	3.92 (4)	3.94 (4)	3.93 (4)	3.95 (4)	3.92 (4)
Tyrosine	1.96 (2)	1.98 (2)	1.97 (2)	1.97 (2)	1.98 (2)
Tryptophan	1.02 (1)	1.01 (1)	1.01 (1)	1.01 (1)	1.02 (1)
H,N(-CH,)-,,COOH	0.97 (1)	–	–	–	–
H,N(-CH,)-,,COOH	–	0.97 (1)	–	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	72	73	74	75	76
Molecular weight by FAB method mass spectrum	3760 (3759.96)	3230 (3229.56)	3358 (3357.73)	3945 (3944.14)	3439 (3439.71)
amino acid composition analysis					
Alanine	2.95 (3)	1.01 (1)	1.00 (1)	1.00 (1)	0.98 (1)
Arginine	4.80 (5)	4.71 (5)	4.72 (5)	4.75 (5)	4.73 (5)
Asparagine	0.90 (1)	0.91 (1)	0.92 (1)	0.93 (1)	0.91 (1)
Aspartic acid	5.09 (5)	1.00 (1)	1.01 (1)	1.01 (1)	1.01 (1)
Glutamic acid	2.05 (2)	2.04 (2)	2.03 (2)	7.22 (7)	2.04 (2)
Glycine	0.99 (1)	–	–	–	0.99 (1)
Histidine	0.98 (1)	0.97 (1)	0.98 (1)	0.97 (1)	0.98 (1)
Leucine	1.98 (2)	1.95 (2)	1.97 (2)	1.97 (2)	1.96 (2)
Lysine	1.01 (1)	2.04 (2)	3.06 (3)	1.01 (1)	1.00 (1)
Proline	1.03 (1)	1.02 (1)	1.01 (1)	1.02 (1)	1.02 (1)
Phenylalanine	2.04 (2)	2.03 (2)	2.03 (2)	2.04 (2)	2.05 (2)
Serine	3.93 (4)	3.94 (4)	3.95 (4)	3.92 (4)	3.93 (4)
Tyrosine	1.96 (2)	1.97 (2)	1.96 (2)	1.97 (2)	1.95 (2)
Tryptophan	1.03 (1)	1.02 (1)	1.03 (1)	1.00 (1)	1.01 (1)
H,N(CH,)-,,COOH	–	–	–	0.99 (1)	–
H,N(CH,)-,,COOH	–	–	–	–	0.96 (1)

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	77	78	79	80	81
Molecular weight by FAB method mass spectrum	3101 (3101.39)	3345 (3343.67)	3673 (3672.97)	3876 (3875.05)	3359 (3357.69)
amino acid composition analysis					
Alanine	0.99 (1)	1.00 (1)	1.00 (1)	2.94 (3)	0.98 (1)
Arginine	4.80 (5)	4.70 (5)	4.71 (5)	4.76 (5)	4.79 (5)
Asparagine	0.91 (1)	0.93 (1)	0.92 (1)	0.91 (1)	0.91 (1)
Aspartic acid	1.00 (1)	2.03 (2)	1.01 (1)	6.08 (6)	0.99 (1)
Glutamine	–	–	–	–	0.94 (1)
Glutamic acid	2.06 (2)	2.04 (2)	5.12 (5)	2.08 (2)	2.04 (2)
Glycine	–	–	1.00 (1)	0.98 (1)	–
Histidine	0.97 (1)	0.98 (1)	0.97 (1)	0.98 (1)	0.98 (1)
Leucine	1.97 (2)	1.97 (2)	1.96 (2)	1.96 (2)	1.96 (2)
Lysine	1.01 (1)	2.03 (2)	2.02 (2)	1.01 (1)	2.03 (2)
Proline	1.02 (1)	1.02 (1)	1.03 (1)	1.02 (1)	1.03 (1)
Phenylalanine	2.03 (2)	2.02 (2)	2.04 (2)	2.03 (2)	2.03 (2)
Serine	3.95 (4)	3.96 (4)	3.91 (4)	3.92 (4)	3.92 (4)
Tyrosine	1.97 (2)	1.98 (2)	1.96 (2)	1.96 (2)	1.97 (2)
Tryptophan	1.01 (1)	1.02 (1)	1.04 (1)	1.00 (1)	1.01 (1)

Note: Figures in parenthesis are theoretical value.

EP 0 426 857 A1

Table 14 (continued)

Example	8 2	8 3	8 4	8 5	8 6
Molecular weight by FAB method mass spectrum	3485 (3485.86)	4074 (4072.27)	3569 (3567.84)	3230 (3229.52)	3471 (3471.80)
amino acid composition analysis					
Alanine	0.97 (1)	1.01 (1)	1.00 (1)	0.99 (1)	0.98 (1)
Arginine	4.71 (5)	4.69 (5)	4.69 (5)	4.81 (5)	4.80 (5)
Asparagine	0.91 (1)	0.92 (1)	0.93 (1)	0.90 (1)	0.91 (1)
Aspartic acid	1.01 (1)	1.00 (1)	1.00 (1)	1.01 (1)	2.03 (2)
Glutamine	0.96 (1)	0.95 (1)	0.95 (1)	0.94 (1)	0.95 (1)
Glutamic acid	2.04 (2)	7.27 (7)	2.04 (2)	2.05 (2)	2.06 (2)
Glycine	–	–	0.99 (1)	–	–
Histidine	0.97 (1)	0.98 (1)	0.98 (1)	0.99 (1)	0.98 (1)
Leucine	1.96 (2)	1.97 (2)	1.97 (2)	1.96 (2)	1.97 (2)
Lysine	3.05 (3)	1.02 (1)	1.00 (1)	1.01 (1)	2.03 (2)
Proline	1.02 (1)	1.02 (1)	1.02 (1)	1.03 (1)	1.03 (1)
Phenylalanine	2.04 (2)	2.02 (2)	2.03 (2)	2.03 (2)	2.04 (2)
Serine	3.94 (4)	3.95 (4)	3.92 (4)	3.91 (4)	3.93 (4)
Tyrosine	1.98 (2)	1.99 (2)	1.96 (2)	1.95 (2)	1.97 (2)
Tryptophan	1.02 (1)	1.01 (1)	1.02 (1)	1.02 (1)	1.02 (1)
$H_2N(CH_2)_{11}COOH$	–	0.97 (1)	–	–	–
$H_2N(CH_2)_{12}COOH$	–	–	0.98 (1)	–	–

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	87	88	89	90	91
Molecular weight by FAB method mass spectrum	3801 (3801.10)	4003 (4003.18)	3544 (3543.90)	3673 (3672.07)	4259 (4258.48)
amino acid composition analysis					
Alanine	0.99 (1)	2.96 (3)	0.97 (1)	0.99 (1)	1.00 (1)
Arginine	4.79 (5)	4.70 (5)	4.78 (5)	4.71 (5)	4.70 (5)
Asparagine	0.92 (1)	0.93 (1)	0.91 (1)	0.89 (1)	0.90 (1)
Aspartic acid	1.01 (1)	6.09 (6)	1.00 (1)	1.02 (1)	1.01 (1)
Glutamine	0.95 (1)	0.96 (1)	0.95 (1)	0.95 (1)	0.96 (1)
Glutamic acid	5.16 (5)	2.04 (2)	2.04 (2)	2.03 (2)	7.21 (7)
Glycine	0.98 (1)	1.00 (1)	–	–	–
Histidine	0.97 (1)	0.98 (1)	0.98 (1)	0.99 (1)	0.98 (1)
Leucine	1.96 (2)	1.97 (2)	1.96 (2)	1.97 (2)	1.98 (2)
Lysine	2.03 (2)	1.01 (1)	2.05 (2)	3.06 (3)	1.02 (1)
Proline	1.03 (1)	1.02 (1)	1.02 (1)	1.02 (1)	1.03 (1)
Phenylalanine	2.01 (2)	2.04 (2)	2.02 (2)	2.04 (2)	2.04 (2)
Serine	3.93 (4)	3.94 (4)	3.91 (4)	3.94 (4)	3.93 (4)
Tyrosine	1.96 (2)	1.98 (2)	1.97 (2)	1.98 (2)	1.95 (2)
Tryptophan	1.01 (1)	1.00 (1)	2.01 (2)	2.04 (2)	2.03 (2)
H,NCH,, COOH	–	–	–	–	0.96 (1)

Note: Figures in parenthesis are theoretical value.

Table 14 (continued)

Example	92	93	94	95	96
Molecular weight by FAB method mass spectrum	3755 (3754.05)	3415 (3415.73)	3659 (3658.01)	3988 (3987.31)	4189 (4189.39)
amino acid composition analysis					
Alanine	1.00 (1)	0.99 (1)	0.98 (1)	1.01 (1)	2.97 (3)
Arginine	4.75 (5)	4.80 (5)	4.74 (5)	4.72 (5)	4.68 (5)
Asparagine	0.91 (1)	0.92 (1)	0.93 (1)	0.90 (1)	0.89 (1)
Aspartic acid	1.01 (1)	1.00 (1)	2.02 (2)	1.01 (1)	6.12 (6)
Glutamine	0.95 (1)	0.95 (1)	0.96 (1)	0.96 (1)	0.96 (1)
Glutamic acid	2.04 (2)	2.03 (2)	2.04 (2)	5.21 (5)	2.03 (2)
Glycine	1.00 (1)	–	–	1.00 (1)	0.99 (1)
Histidine	0.97 (1)	0.98 (1)	0.97 (1)	0.98 (1)	0.98 (1)
Leucine	1.96 (2)	1.97 (2)	1.96 (2)	1.97 (2)	1.97 (2)
Lysine	1.00 (1)	1.00 (1)	2.03 (2)	2.03 (2)	1.03 (1)
Proline	1.02 (1)	1.01 (1)	1.02 (1)	1.03 (1)	1.03 (1)
Phenylalanine	2.03 (2)	2.02 (2)	2.03 (2)	2.02 (2)	2.02 (2)
Serine	3.92 (4)	3.90 (4)	3.89 (4)	3.91 (4)	3.92 (4)
Tyrosine	1.96 (2)	1.95 (2)	1.99 (2)	1.97 (2)	1.96 (2)
Tryptophan	2.02 (2)	2.03 (2)	2.04 (2)	2.01 (2)	2.03 (2)
$H_2N(CH_2)_2COOH$	0.99 (1)	–	–	–	–

Note: Figures in parenthesis are theoretical value.

Example 97

10 g of cellulose particles (sold by Seikagaku Kogyo Co., Ltd., CM-Cellulofine CH) were suspended in

50 ml of anhydrous dioxane (obtained by distilling dioxane commercially available in the presence of metallic sodium) and to the resulting suspension were added 0.5 g of N-hydroxysuccinimide and 1.0 g of dicyclohexyl carbodiimide and then the mixture was shaken at room temperature overnight. The resulting mixture was washed with 0.02 mole/liter of a phosphate buffer solution (pH: 7.4) and filtered with suction. The resulting particles were admixed with 0.02 mole/liter of a phosphate buffer solution (pH: 7.4, 20 ml) containing 20 mg of the peptide obtained in Example 1 and the mixture was stirred at 4° C overnight. The mixture was filtered with suction. Although the filtrate was subjected to analytical reversed phase high performance liquid chromatography, the remaining unreacted peptide was not observed (immobilization degree of peptide on carrier: about 100%). Like this, about 10 g of the adsorbent wherein 20 mg of the peptide obtained in Example 1 was immobilized on cellulose particles was obtained.

Example 98

According to the same manner as that described in Example 97, about 10 g of an adsorbent wherein 18.4 mg of the peptide obtained in Example 9 was immobilized on polyvinyl alcohol particles (immobilization degree of peptide on carrier: about 92%) was obtained except that 10 g of polyvinyl alcohol particles (manufactured by Toso Co., Ltd., Tsk-gel CM-Toyopearl 650C) were used in place of 10 g of cellulose particles and 20 mg of the peptide obtained in Example 9 was used in place of 20 mg of the peptide obtained in Example 1.

Example 99

10 g of porous glass particles (manufactured by Electro-nucleonics Corp., U.S.A., CPG-10-1000) were heated under reflux in 100ml of a toluene solution containing 5 ml of γ-aminopropyltriethoxysilane for 24 hours. The resulting mixture was washed with anhydrous dioxane and filtered with suction. The resulting particles were suspended in 100 ml of anhydrous dioxane and to the suspension was added 3 g of succinic anhydride. Then the mixture was stirred at room temperature overnight. The resulting mixture was washed with anhydrous dioxane and filtered with suction. The resulting particles were suspended in 50 ml of anhydrous dioxane and to the suspension was added 0.5 g of N-hydroxysuccinimide and 1.0 g of dicyclohexylcarbodiimide. The mixture was stirred at room temperature overnight. The resulting mixture was washed with 0.02 mole/liter of a phosphate buffer solution (pH: 7.4) and filtered with suction. The resulting particles were admixed with 0.02 mole/liter of a phosphate buffer solution containing 20 mg of the peptide obtained in Example 17 (pH: 7.4, 20 ml) and this mixture was stirred at 4° C overnight. The mixture was filtered with suction to obtain about 10 g of an absorbent wherein 20 mg of the peptide obtained in Example 17 was immobilized on the porous glass particle (immobilization degree of peptide on carrier: about 100%).

Examples 100 to 192

According to any one of manners as those described in Examples 97 to 99, adsorbents wherein peptides were immobilized on the granular carriers were obtained except that 20 mg of the peptides shown in Table 10 were used. The granular carriers used and immobilization degrees of peptide on carriers are shown in Table 15, respectively.

Table 15

Example	Peptide	Granular carrier	Immobilization degree (%)
100	obtained in Example 2	cellulose particles	about 98
101	" Example 3	"	" 90
102	" Example 4	"	" 85
103	" Example 5	"	" 75
104	" Example 6	"	" 92
105	" Example 7	polyvinyl alcohol particles	" 95
106	" Example 8	cellulose particles	" 95
107	" Example 10	polyvinyl alcohol particles	" 100
108	" Example 11	"	" 98
109	" Example 12	cellulose particles	" 92
110	" Example 13	"	" 80
111	" Example 14	"	" 88
112	" Example 15	"	" 90
113	" Example 16	"	" 92
114	" Example 18	"	" 100

Table 15 (continued)

Example	Peptide	Granular carrier	Immobilization degree (%)
115	obtained in Example 19	cellulose particles	about 95
116	" Example 20	"	" 93
117	" Example 21	porous glass particles	" 85
118	" Example 22	"	" 100
119	" Example 23	"	" 100
120	" Example 24	cellulose particles	" 94
121	" Example 25	porous glass particles	" 98
122	" Example 26	cellulose particles	" 100
123	" Example 27	"	" 96
124	" Example 28	"	" 96
125	" Example 29	"	" 92
126	" Example 30	"	" 96
127	" Example 31	"	" 99
128	" Example 32	"	" 93
129	" Example 33	"	" 100
130	" Example 34	"	" 98

Table 15 (continued)

Example	Peptide	Granular carrier	Immobilization degree (%)
131	obtained in Example 35	cellulose particles	about 90
132	" Example 36	"	" 85
133	" Example 37	"	" 75
134	" Example 38	"	" 92
135	" Example 39	polyvinyl alcohol particles	" 95
136	" Example 40	cellulose particles	" 95
137	" Example 41	polyvinyl alcohol particles	" 92
138	" Example 42	"	" 100
139	" Example 43	"	" 98
140	" Example 44	cellulose particles	" 92
141	" Example 45	"	" 80
142	" Example 46	"	" 88
143	" Example 47	"	" 90
144	" Example 48	"	" 92
145	" Example 49	"	" 100

Table 15 (continued)

Example	Peptide	Granular carrier	Immobilization degree (%)
146	obtained in Example 50	cellulose particles	about 100
147	" Example 51	"	" 95
148	" Example 52	"	" 93
149	" Example 53	porous glass particles	" 85
150	" Example 54	"	" 100
151	" Example 55	"	" 100
152	" Example 56	cellulose particles	" 94
153	" Example 57	"	" 100
154	" Example 58	"	" 92
155	" Example 59	"	" 92
156	" Example 60	"	" 90
157	" Example 61	"	" 90
158	" Example 62	"	" 95
159	" Example 63	polyvinyl alcohol particles	" 96
160	" Example 64	"	" 89
161	" Example 65	porous glass particles	" 98

Table 15 (continued)

Example	Peptide	Granular carrier	Immobilization degree (%)
162	obtained in Example 66	porous glass particles	about 100
163	" Example 67	polyvinyl alcohol particles	" 90
164	" Example 68	"	" 88
165	" Example 69	"	" 93
166	" Example 70	cellulose particles	" 95
167	" Example 71	"	" 94
168	" Example 72	"	" 88
169	" Example 73	"	" 99
170	" Example 74	"	" 100
171	" Example 75	porous glass particles	" 98
172	" Example 76	polyvinyl alcohol particles	" 91
173	" Example 77	"	" 85
174	" Example 78	cellulose particle	" 93
175	" Example 79	"	" 96
176	" Example 80	"	" 85

Table 15 (continued)

Example	Peptide	Granular carrier	Immobilization degree (%)
177	obtained in Example 81	cellulose particles	about 98
178	" Example 82	"	" 100
179	" Example 83	polyvinyl alcohol particles	" 88
180	" Example 84	"	" 85
181	" Example 85	"	" 82
182	" Example 86	cellulose particles	" 99
183	" Example 87	"	" 95
184	" Example 88	"	" 90
185	" Example 89	porous glass particles	" 98
186	" Example 90	polyvinyl alcohol particles	" 100
187	" Example 91	"	" 90
188	" Example 92	cellulose particles	" 88
189	" Example 93	"	" 89
190	" Example 94	"	" 94
191	" Example 95	"	" 98
192	" Example 96	"	" 89

Experiment 1

Obtaining IL-6 receptor expression cells

A human lymphocyte fraction was obtained from human peripheral blood with Ficoll-Paque (manufactured by Pharmacia-LKB). The fraction was reacted with a cultured supernatant of Epstein-Barr Virus producing cell strain B95-8 to transform the human lymphocytes to obtain IL-6 receptor expression cells.

Preparation of FITC labeled anti-IL-6 antibody

1 mg of anti-human IL-6 antibody [Rabbit Anti-human Interleukin-6, manufactured by Genzyme Corp.] was dissolved in a 0.05 M carbonate buffer solution (pH: 9.5) and to the resulting solution was added 10 μg of FITC [Fluorocein isothiocyanate, manufactured by Sigma Corp.]. The mixture was stirred at 4 °C overnight. The resulting solution was passed through PD-10 column (manufactured by Pharmacia-LKB) to obtain FITC labeled anti-IL-6 antibody as a firstly eluted fraction.

Activity for inhibition of binding of IL-6 to receptor by peptide

10^5 IL-6 receptor expression cells were suspended in 0.5 % BSA (bovine serum albumin, manufactured by Sigma Corp.)-PBS (a phosphate buffer solution containing 0.15 M sodium chloride, pH 7.4) and to the resulting suspension were added 50 ng of IL-6 [Human recombinant interleukin-6, manufactured by Genzyme Corp.) and 5 μg of the peptide obtained in any one of Examples 1 to 96. The suspension was allowed to stand at 4° C for one hour. Then, after washing the cells centrifugally with 0.5% BSA-PBS three times (1200 rpm, 5 min.), 1 μg of FITC labeled anti-IL-6 antibody was added and the mixture was allowed to stand at 4° C for 30 minutes. After washing with 0.5% BSA-PBS three times, the intensity of fluorescence of the cells was determined. The binding inhibition activity of the peptide of each Example was evaluated, by taking the value obtained without addition of the peptide as a control and taking the value obtained without addition of IL-6 as a blank, according to the following formula:

$$\text{Binding inhibition activity (\%)} = \left(\frac{\text{Intensity of fluorescence of control} - \text{Intensity of fluorescence when peptide being added}}{\text{Intensity of fluorescence of control} - \text{Intensity of fluorescence of blank}} \right) \times 100$$

The results obtained for the peptides of Examples 1 to 96 are shown in Table 16.

Table 16

Peptide	Binding inhibition activity (%)
peptide obtained in Example 1	38
" Example 2	35
" Example 3	40
" Example 4	42
" Example 5	28
" Example 6	36
" Example 7	38
" Example 8	37
" Example 9	30
" Example 10	28
" Example 11	34
" Example 12	35
" Example 13	25
" Example 14	32
" Example 15	30
" Example 16	31
" Example 17	60

Table 16 (continued)

Peptide	Binding inhibition activity (%)
peptide obtained in Example 18	55
" Example 19	62
" Example 20	65
" Example 21	52
" Example 22	58
" Example 23	58
" Example 24	55
" Example 25	45
" Example 26	42
" Example 27	48
" Example 28	47
" Example 29	41
" Example 30	46
" Example 31	48
" Example 32	44
" Example 33	58
" Example 34	55

Table 16 (continued)

Peptide		Binding inhibition activity (%)
peptide obtained in	Example 35	60
"	Example 36	62
"	Example 37	48
"	Example 38	56
"	Example 39	58
"	Example 40	37
"	Example 41	30
"	Example 42	28
"	Example 43	34
"	Example 44	35
"	Example 45	25
"	Example 46	32
"	Example 47	30
"	Example 48	31
"	Example 49	30
"	Example 50	25
"	Example 51	32

Table 16 (continued)

Peptide		Binding inhibition activity (%)
peptide obtained in	Example 52	35
"	Example 53	22
"	Example 54	28
"	Example 55	28
"	Example 56	25
"	Example 57	63
"	Example 58	60
"	Example 59	60
"	Example 60	58
"	Example 61	45
"	Example 62	50
"	Example 63	51
"	Example 64	55
"	Example 65	68
"	Example 66	65
"	Example 67	51
"	Example 68	55

Table 16 (continued)

Peptide		Binding inhibition activity (%)
peptide obtained in	Example 69	54
"	Example 70	59
"	Example 71	60
"	Example 72	57
"	Example 73	71
"	Example 74	70
"	Example 75	63
"	Example 76	58
"	Example 77	58
"	Example 78	60
"	Example 79	62
"	Example 80	59
"	Example 81	76
"	Example 82	70
"	Example 83	63
"	Example 84	65
"	Example 85	60

Table 16 (continued)

Peptide	Binding inhibition activity (%)
peptide obtained in Example 86	58
" Example 87	72
" Example 88	66
" Example 89	68
" Example 90	71
" Example 91	60
" Example 92	62
" Example 93	59
" Example 94	63
" Example 95	65
" Example 96	52

Experiment 2

Preparation of biotin labeled anti-IL-6 antibody

200 μg of the same anti-IL-6 antibody as that used in Experiment 1 was dissolved in 0.2 ml of a 0.1 M $NaHCO_3$ aqueous solution. To the resulting solution was added 20 μg of a solution of NHS-LS-biotin [manufactured by Pierce Corp.] in DMF (1 mg/ml) and the mixture was allowed to react at room temperature for 4 hours. The reaction mixture was dialyzed to PBS at 4° C to obtain a biotin labeled anti-IL-6 antibody.

Adsorption of IL-6 in serum

50 mg of the adsorbent obtained in any one of Examples 97 to 192 was shaken with 500 μl of serum from a patient with rheumatism containing IL-6 at 37° C for 3 hours and the supernatant was used as a test solution.

Measurement of IL-6 concentration in test solution and evaluation of adsorbability of adsorbent

The same anti-IL-6-antibody as that used in Experiment 1 was immobilized in each well of a flat bottom 96 well-plate [Falcon Rigid-Assay Plate, manufactured by Becton Dickinson Corp.] in an amount of 2.5 μg/w ll. After blocking each well with 1% BSA-PBS, 50 μl portions of the test solution were distributed into wells. After standing at 4° C overnight, each well was washed and 0.5 μl portions of biotin labeled anti-IL-6 antibody were distributed into wells. The plate was further allowed to stand at 37° C for one hour. After washing each well, HRP labeled streptoavidin [1500-fold dilution, manufactured by Kirkegaard & Perry Lab. Inc.] was distributed into each well and the plate was further allowed to stand at 37° C for 30 minutes. After washing each well, ABTS was added in the presence of H_2O_2 to develop color and difference between

absorbances at 409 nm and 501 nm of each well was measured. A calibration curve was prepared from the absorbances of wells wherein solutions containing a known concentration of human IL-6 were added in place of the test solution and IL-6 concentration in each test solution was determined by using the calibration curve. An adsorption removal rate of IL-6 was calculated by using the IL-6 concentration obtained by using an adsorbent, wherein glycine was immobilized on cellulose particles in place of peptide as a control value, according to the following formula:

$$\text{Adsorption removal rate (\%)} = \left(\frac{\text{Control value} - \text{Concentration in test solution}}{\text{Control value}} \right) \times 100$$

The results are shown in Table 17.

Table 17

Absorbent	Adsorption removal rate (%)
obtained in Example 97	60
" Example 98	52
" Example 99	85
" Example 100	58
" Example 101	55
" Example 102	57
" Example 103	50
" Example 104	53
" Example 105	59
" Example 106	50
" Example 107	55
" Example 108	53
" Example 109	45
" Example 110	54
" Example 111	54
" Example 112	49
" Example 113	82

Table 17 (continued)

Absorbent	Adsorption removal rate (%)
obtained in Example 114	80
" Example 115	83
" Example 116	82
" Example 117	72
" Example 118	84
" Example 119	81
" Example 120	79
" Example 121	65
" Example 122	63
" Example 123	68
" Example 124	70
" Example 125	58
" Example 126	62
" Example 127	65
" Example 128	63
" Example 129	60
" Example 130	58

Table 17 (continued)

Absorbent		Adsorption removal rate (%)
obtained in	Example 131	55
	Example 132	57
"	Example 133	50
"	Example 134	53
"	Example 135	39
"	Example 136	30
"	Example 137	52
"	Example 138	35
"	Example 139	33
"	Example 140	25
"	Example 141	34
"	Example 142	24
"	Example 143	29
"	Example 144	32
"	Example 145	85
"	Example 146	30
"	Example 147	33

Table 17 (continued)

Absorbent		Adsorption removal rate (%)
obtained in	Example 148	32
"	Example 149	22
"	Example 150	34
"	Example 151	31
"	Example 152	29
"	Example 153	81
"	Example 154	79
"	Example 155	75
"	Example 156	79
"	Example 157	66
"	Example 158	71
"	Example 159	68
"	Example 160	75
"	Example 161	86
"	Example 162	85
"	Example 163	70
"	Example 164	78

Table 17 (continued)

Absorbent	Adsorption removal rate (%)
obtained in Example 165	72
" Example 166	80
" Example 167	79
" Example 168	77
" Example 169	90
" Example 170	88
" Example 171	81
" Example 172	79
" Example 173	77
" Example 174	78
" Example 175	81
" Example 176	77
" Example 177	93
" Example 178	89
" Example 179	81
" Example 180	83
" Example 181	77

Table 17 (continued)

Absorbent	Adsorption removal rate (%)
obtained in Example 182	77
" Example 183	72
" Example 184	66
" Example 185	88
" Example 186	90
" Example 187	78
" Example 188	81
" Example 189	79
" Example 190	82
" Example 191	86
" Example 192	75

INDUSTRIAL APPLICABILITY

According to the present invention, there is provided a peptide of the general formula (I) useful in the treatment of autoimmune disease. Since the peptide of the general formula (I) inhibits binding of IL-6 to its receptor, administration of the peptide to a patient with an autoimmune disease is effective for inhibiting the production of autoimmune antibody caused by binding of IL-6 to its receptor.

Further, according to the present invention, there is provided an adsorbent wherein the peptide of the general formula (I) is immobilized on an insoluble carrier. The absorbent can be used for removing IL-6 from a patient with an autoimmune disease by extracorporeal blood circulation system using the absorbent.

Claims

1. A peptide being capable of binding to interleukin 6 represented by the general formula:
H-X-A-Y-Z
wherein A is a peptide segment formed by binding 6 to 50 amino acids; each of X and Y is a single bond or an amino acid residue selected from the group consisting of Asp, Glu, Lys, Ala and a divalent group of the formula: $-NH(CH_2)_n-CO-$(wherein n is an integer of 1 to 17), or a peptide segment composed of 2 to 10 amino acid residues selected from the above group bound to each other through a peptide bond; Z is hydroxyl group or amino group.

2. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Gly-Thr-Val-His-Leu-Leu-Val-Asp-Val-Pro-Pro-Glu-Glu-Pro-Gln-Leu-Ser-Cys-Phe-Arg-Lys-.

3. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Arg-Lys-Phe-Gln-Asn-Ser-Pro-Ala-Glu-Asp-Phe-Gln-Glu-Pro-Cys-Gln-Tyr-Ser-Gln-Glu-Ser-.

4. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Thr-Ser-Leu-Pro-Gly-Asp-Ser-Val-Thr-Leu-Thr-Cys-Pro-Gly-Val-Glu-Pro-Glu-Asp-.

5. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Gln-Ala-Leu-Thr-Thr-Asn-Lys-Asp-Asp-Asp-Asn-Ile-Leu-Phe-Arg-Asp-Ser-Ala-.

6. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys.

7. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Ser-Thr-Pro-Ser-Leu-Thr-Thr-Lys-Ala-Val-Leu-Leu-Val-Arg-Lys-Phe-Gln-Asn-Ser-Pro-Ala-Glu-Asp-.

8. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Asn-Pro-Arg-Trp-Leu-Ser-Val-Thr-Trp-Gln-Asp-Pro-His-Ser-.

9. A peptide according to claim 1, wherein A is a peptide segment of the formula: -His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys.

10. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-.

11. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Asp-Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-.

12. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Gln-Asp-Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-.

13. A peptide according to claim 1, wherein A is a peptide segment of the formula: -Trp-Gln-Asp-Pro-His-Ser-Trp-Asn-Ser-Ser-Phe-Tyr-Arg-Leu-Arg-Phe-Glu-Leu-Arg-Tyr-Arg-Ala-Glu-Arg-Ser-Lys-.

14. An adsorbent comprising the peptide according to claim 1 immobilized on a carrier.

15. An adsorbent comprising the peptide according to claim 2 immobilized on a carrier.

16. An adsorbent comprising the peptide according to claim 3 immobilized on a carrier.

17. An adsorbent comprising the peptide according to claim 4 immobilized on a carrier.

18. An adsorbent comprising the peptide according to claim 5 immobilized on a carrier.

19. An adsorbent comprising the peptide according to claim 6 immobilized on a carrier.

20. An adsorbent comprising the peptide according to claim 7 immobilized on a carrier.

21. An adsorbent comprising the peptide according to claim 8 immobilized on a carrier.

22. An adsorbent comprising the peptide according to claim 9 immobilized on a carrier.

23. An adsorbent comprising the peptide according to claim 10 immobilized on a carrier.

24. An adsorbent comprising the peptide according to claim 11 immobilized on a carrier.

25. An adsorbent comprising the peptide according to claim 12 immobilized on a carrier.

26. An adsorbent comprising the peptide according to claim 13 immobilized on a carrier.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP90/00142

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] C07K7/08, 7/10, 17/08, 17/10, 17/14, A61K37/02, B01J20/22, G01N33/564, 33/566

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C07K7/08, 7/10, 17/00, A61K37/02, B01J20/22, G01N33/564, 33/566

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Science (Washington, D.C., 1883 -), Vol. 241, No. 4867 (1988), Katsuhiko Yamasaki, et al. 「Cloning and Expression of the Human Interleukin - 6 (BSF - 2/IFNβ 2) Receptor」, pp. 825 - 828	1 - 26
Y	Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. Vol. 64, No. 7 (1988), Katsuhiko Yamasaki, et al. 「Molecular Structure of Interleukin 6 Receptor」 pp. 209 - 211	1 - 26
Y	JP, A, 63-159396 (F. Hoffmann-La Roche & Co., A.G.), 2 July 1988 (02. 07. 88), Pages 2 to 4 & EP, A, 263529	1 - 26
Y	JP, A, 63-154700 (Asahi Chemical Industry Co., Ltd.), 27 June 1988 (27. 06. 88), Pages 2 to 3 (Family: none)	1 - 26

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
April 23, 1990 (23. 04. 90)	May 7, 1990 (07. 05. 90)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	

FURTHER INFORMATION CONTINUED FR M THE SECOND SHEET

Y	JP, A, 63-152396 (Scripps Clinic & Research Foundation), & EP, A, 255206	1 - 26
Y	JP, A, 62-281897 (K.K. Daiichi Radioisotope Kenkyusho), 7 December 1987 (07. 12. 87), Pages 1 to 6 & DE, A, 3725861	1 - 26

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.